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Intravenous iron increases labile serum iron but does not impair forearm blood flow reactivity in dialysis patients

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Background. There are concerns about adverse vascular effects of intravenous iron by inducing oxidative stress. We therefore examined the effect of a single high dose of intravenous iron on endothelial function and biochemical markers of iron homeostasis.

Methods. In a randomized, placebo-controlled, double-blind, parallel-group study, forearm blood flow (FBF) was assessed by strain-gauge plethysmography in 38 peritoneal dialysis patients before and after a single intravenous infusion of 300 mg iron sucrose.

Results. Iron infusion increased total (Δ 601 μg/100 mL, CI 507, 696) and non–transferrin-bound iron (Δ 237.2 μmol/L, CI 173.6, 300.8) approximately 10-fold, as well as redox-active iron nearly five-fold (Δ 0.76 μmol/L, CI 0.54, 0.98). After iron infusion basal FBF was 59% higher than after placebo. FBF response to acetylcholine before and after iron infusion was 263 ± 32% and 310 ± 33%, corresponding to 304 ± 43% and 373 ± 29% in the placebo group, respectively. Before and after iron or placebo infusion, glyceryl-trinitrate increased resting FBF to 232 ± 22% and 258 ± 21% in the iron group, and to 234 ± 18% and 270 ± 30% in the placebo group. L-N-monomethyl-arginine decreased FBF to 70 ± 4% and 72 ± 3% before and after iron, and to 74 ± 4% and 73 ± 4% before and after placebo infusions, respectively. Despite higher basal FBF after iron infusion, absolute and relative FBF changes in response to vasoactive substances were not significantly different between iron and placebo groups.

Conclusion. Our data suggest that 300 mg intravenous iron sucrose has a vasodilatory effect, but does not impair vascular reactivity in dialysis patients, despite a significant increase in non–transferrin-bound and redox-active iron.

Sufficient iron substitution is critical for adequate treatment of anemia, especially in erythropoietin-treated renal failure patients [1–4]. Because oral iron is poorly absorbed, intravenous iron supplementation is preferred in most of these patients [5]. While the prevalence of iron deficiency is lower in peritoneal dialysis (PD) compared to hemodialysis (HD) patients, PD patients receive higher bolus doses of iron at longer intervals since they only visit the center every four to six weeks. Furthermore, unlike HD patients, PD patients often have limited venous access and their forearm veins need to be kept permeable. When infused over a period of two hours, iron sucrose in single doses of up to 300 mg has been well tolerated in dialysis patients [6]. There are, however, concerns about intravenous iron administration. A single bolus of 300 mg of intravenous iron sucrose has been reported to depress phagocyte function in PD patients [7]. Impairment of neutrophil function is also present in chronic HD patients receiving intravenous iron therapy, especially in those with ferritin levels above 650 μg/L [8]. Therefore, high-dose or long-term intravenous iron administration may be associated with an increased risk of infection [9]. Furthermore, increased blood levels of non–transferrin-bound iron (NTBI) or its redox-active part may cause endothelial dysfunction and platelet activation by generation of reactive oxygen species, inactivation of endothelium-derived nitric oxide (NO), and oxidation of low-density lipoproteins (LDL) [10–12]. These are important mechanisms in the pathogenesis of atherosclerosis [13].

To date, there are no sufficient studies available that have addressed the effect of high dose intravenous iron on endothelial function and redox-active iron. We therefore investigated whether one single intravenous dose of 300 mg of iron sucrose acutely deteriorates vascular function in stable PD patients.

METHODS

The study was approved by the Ethics Committee of the Medical University Vienna and conforms with the

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principles outlined in the Declaration of Helsinki, including current revisions and the European Guidelines on Good Clinical Practice.

Study population

Thirty-eight patients treated with continuous ambulatory (CAPD, $N = 16$) or automated (APD, $N = 22$) peritoneal dialysis at the Medical University Vienna, from whom written informed consent was obtained before enrollment, were included consecutively in order of attendance over a period of seven months. Patients were 18 years of age or older, had been stable on PD for at least three months, and without an episode of peritonitis for at least 2 months before inclusion. Furthermore, none of the patients received oral or intravenous iron preparations for at least six weeks before study begin. Erythropoietin-treated patients were included if hemoglobin levels were ≤14 g/100 mL and ferritin ≤500 μg/L. Patients without erythropoietin therapy were included if hemoglobin was ≤12 g/100 mL and ferritin ≤500 μg/L, or with hemoglobin between 12 and 14 g/100 mL if ferritin levels were below 200 μg/L.

Exclusion criteria were participation in another trial within 4 weeks before study begin, allergy or intolerance to iron sucrose, poor general condition of health, acute infection, acute vascular disease (history of stroke, myocardial infarction, or peripheral occlusive disease within three months before start of the study), and pregnancy. Coronary artery disease was defined as history of percutaneous or surgical revascularization and/or myocardial infarction. Accordingly, peripheral vascular disease and cerebrovascular arterial disease were defined as history of percutaneous or surgical revascularization and/or gangrene or cerebral stroke, respectively. Hypertension was defined as blood pressure higher than 140/90 mm Hg and/or use of antihypertensive medication. Study groups were well balanced with respect to concomitant diseases. Arterial hypertension was the most common medical history ($N = 18$ in both study groups), followed by coronary heart disease ($N = 4$ in both patient groups) and diabetes mellitus ($N = 3$ in iron group, $N = 4$ in placebo group). No patient had a history of peripheral vascular disease. One patient in the iron group and two patients in the placebo group had a history of cerebrovascular disease. Smoking (3 and 5 subjects) and statin therapy (8 and 11 subjects) were equally distributed between groups. One subject receiving iron and six subjects receiving placebo reported intake of chronic vitamin supplementation (low dose folate and/or vitamin B_{12} and/or vitamin B_6). All patients except one in the iron group and two in the placebo group received erythropoietin-beta or darbepoetin-alfa subcutaneously. For detailed patient characteristics see Table 1.

Smokers were asked not to smoke for 12 hours before and during the study day. Patients were asked to fast at least for 10 hours before measurement of vascular function. All subjects continued their regular medication. Studies were conducted in a quiet room with an ambient temperature of 22°C with complete resuscitation facilities.

Table 1. Baseline characteristics for PD patients receiving 300 mg intravenous iron sucrose or placebo infusion

	Iron ($N = 19$)	Placebo ($N = 19$)
Age *years*	57 ± 11	56 ± 14
Sex *m/f*	15/4	13/6
BMI kg/m^2	26.8 ± 3.1[a]	23.9 ± 3.8
CAPD *N*	9	7
APD *N*	10	12
Duration of PD *months*	16 ± 11	20 ± 16
RRF *mL/min*	6.10 ± 2.79	6.19 ± 3.73
Kt/V_{urea}	2.59 ± 0.56	2.72 ± 0.76

BMI, body mass index; CAPD, continuous ambulatory peritoneal dialysis; APD, automated peritoneal dialysis; PD, peritoneal dialysis; RRF, residual renal function; Kt/V_{urea}, weekly urea clearance over total body water. Mean ± SD.
[a] $P < 0.05$ vs. placebo.

Outcomes

The primary end point was forearm blood flow (FBF) reactivity in PD patients after intravenous infusion of 300 mg of iron sucrose compared to placebo administration.

Secondary end points were effect on serum concentrations of total iron, non–transferrin-bound iron, and redox-active iron after intravenous iron infusion compared to placebo infusion.

Forearm blood flow measurements

Forearm blood flow was measured in both arms, as described previously [14, 15]. Briefly, strain gauges, placed on the forearms, were connected to plethysmographs (EC-6, D.E. Hokanson, Bellevue, WA, USA) to measure changes in forearm volume in response to inflation of venous congesting cuffs. Drug effects were expressed as the ratio of blood flow in the intervention to the control arm [15, 16], where predose ratio was defined as 100%. Wrist cuffs were inflated to suprasystolic pressures during each measurement to exclude circulation to the hands. Flow measurements were recorded for 9 seconds at 30-second intervals during drug infusions.

Study design

The study followed a prospective, randomized, double-blind, placebo-controlled, parallel-group design. Patients were randomized into one of the following two groups: patients of the iron group ($N = 19$) received a single dose infusion of 300 mg of iron sucrose (Venofer, Vifor, Levallois-Perret, France) in 50 mL 0.9% sodium chloride solution over two hours. Patients of the placebo group

($N = 19$) received 50 mL vehicle alone. Measurements of endothelial function were done identically in both groups, before and immediately after iron/placebo infusion. In both patient groups, blood samples were taken at baseline and after infusion. Additionally, 24-hour urine and dialysate samples were taken for clearance measurements before start of the study.

A sample size calculation was carried out prior to the study. To detect a 75% difference in vascular function between iron sucrose or placebo, and assuming a type I error of 0.05, a power of 80%, and a dropout of two patients per group, a sample size of 38 patients (19 per group) was estimated.

Randomization and double-blinding

The random allocation sequence was generated using the web site www.randomization.com. Patients were enrolled by one of the investigators. Infusions were prepared in black nontransparent syringes and infusion lines by study nurses not involved in administration of drugs or measurement of vascular function. Infusions of iron and placebo were given by study nurses. Nurses and investigators who performed infusions and/or measurements of vascular function as well as patients and laboratory personnel were blinded to group assignment.

Experimental protocol

A fine-bore needle (27G needle Sterican; B. Braun, Melsungen, Germany) was inserted into the brachial artery of the left arm for the administration of vasodilators. After a 10-minute resting period, FBF during infusion of 0.9% saline was measured for five minutes, followed by measurements of FBF response to incremental doses of the endothelium-dependent dilator acetylcholine (ACh, Clinalfa, Läufelfingen, Switzerland; 25, 50, and 100 nmol/min for 3 minutes per dose level). After a 15-minute washout period with intra-arterial saline infusions to allow restoration of predose blood flow, the response to the endothelium-independent dilator glyceryl-trinitrate (GTN, Perlinganit®; Gebro Pharma, Fieberbrunn, Austria; 4, 8, 16 mmol/min for 3 minutes per dose level) was measured. After another 15-minute washout period, this was repeated for the endothelium-dependent vasoconstrictor and NO-synthase inhibitor L-N-monomethyl-arginine (L-NMMA; Clinalfa, 1, 2, 4 μmol/min for 3 minutes per dose level).

Subsequently, a plastic cannula was inserted into an antecubital or dorsal hand vein for a baseline blood draw. The cannula was then used for administration of iron sucrose or placebo over 120 minutes. Immediately after the end of infusion, blood was collected from the other arm by a fresh venipuncture.

Forearm blood flow responses to endothelium-dependent and endothelium-independent dilators and to L-NMMA were repeated in a manner identical to the measurements obtained before iron sucrose or placebo infusions.

As safety parameters, arterial blood pressure and pulse rate were recorded.

Measurement of mobilizer-dependent NTBI and redox-active iron

Mobilizer-dependent non–transferrin-bound iron was measured according to the method published by Breuer et al [17], using desferrioxamine (DFO, Desferal®; Novartis, Vienna, Austria) as iron chelator and oxalate as iron mobilizing agent. The binding of NTBI from the sera to DFO-coated wells was detected with a nonfluorescent iron-calcein complex. Calcein has a lower affinity to iron than DFO and can donate iron to DFO not occupied by NTBI from the serum of patients. Therefore, calcein becomes iron free. Fluorescence of iron-free calcein was measured in a fluorescence multiwell plate reader (Victor^2 from Wallac, Turku, Finland—now Perkin-Elmer, Boston, MA, USA) with excitation/emission filters of 485/530 nm.

Redox-active iron was measured with slight modifications as described by Esposito et al [18], based on the principle that dichlorofluorescine (DCF; Molecular Probes, Inc., Eugene, OR, USA) is converted from its nonfluorescent to fluorescent form by several oxidants. In brief, patient sera (20 μL) were transferred in quadruplicates to black, clear-bottom 96-well plates (Greiner, Bio-one, Graz, Austria). Two wells were incubated with iron free HEPES buffered saline (HBS) (20 mmol/L HEPES, 150 mmol/L NaCl, pH 7.4; Merck, Darmstadt, Germany) containing 150 μmol/L ascorbate and 5 μmol/L DCF at 37°C in the dark. The other two wells were incubated with 180 μL of the same solution containing 50 μmol/L of the iron chelator Deferiprone (L1; kindly provided by Dr. Peter Nielsen, UKE, Hamburg, Germany). HBS was rendered iron free by treatment with 1 g/100 mL Chelex-100 (Sigma-Aldrich, Vienna, Austria). The kinetics of fluorescence increase were measured in the fluorescence multiwell plate reader. Measurements between 60 and 120 minutes were used to calculate slopes (r) of DCF fluorescence intensity over time. The fluorescence increase measured in presence of L1 represents oxidation of DCF by several other oxidants (e.g., peroxidases or hypochlorous acid generated by myeloperoxidases). Therefore, the difference in the rate of oxidation of DCF with and without addition of the chelator L1 represents the redox-active component of NTBI. The duplicate values of r with and without addition of L1 were averaged, and redox-active iron (in μmol/L) was determined from calibration curves correlating the difference in slopes with and without L1 against the iron concentration.

Other biochemical methods

Plasma samples for non–transferrin-bound and redox-active iron were taken at baseline (before start of infusion) and after 120 minutes (immediately after end of infusion). Plasma samples were batch analyzed. Complete blood counts as well as blood, dialysate, and urine chemistry were performed by standard procedures in an ISO 9001:2000 certified laboratory. Serum iron was measured using a Hitachi 747 analyzer (Roche Diagnostics, Mannheim, Germany). Serum transferrin concentrations were determined by the Behring Nephelometer II analyzer (Dade Behring, Liederbach, Germany). Serum ferritin levels were analyzed with a Hitachi 911 instrument (Roche Diagnostics). Transferrin saturation was calculated as follows: serum iron (μg/100 mL)/serum transferrin (mg/100mL) × 70.9. Concentrations of total homocysteine (tHcy) were measured by a fluorescence polarization immunoassay (IMx® Analyzer; Abbott, Wiesbaden, Germany).

Statistical methods

All data were tested for normal distribution and compared using Student paired or unpaired *t* test, if appropriate. Data with log-normal distribution were compared using the Mann Whitney *U* test. Forearm blood flow measurements were calculated as mL/min/100 mL forearm volume and expressed as percent change from predose ratio. The effects on intra-arterial ACh, GTN, and L-NMMA were assessed by analysis of variance (ANOVA) for repeated measurements, followed by Bonferroni corrected *t* tests. Statistical analysis was carried out using the Statistica software package (release 6.0; StatSoft, Inc., Tulsa, OK, USA). $P < 0.05$ was considered significant. Values are expressed as mean ± SEM unless indicated otherwise.

RESULTS

Laboratory results

Baseline laboratory values were comparable between study groups, with the exception of calcium × phosphorous ion product (Ca×P) and LDL cholesterol, which were higher in subjects receiving iron. For detailed results see Table 2.

Table 2. Baseline laboratory parameters for PD patients receiving 300 mg intravenous iron sucrose or placebo infusion

	Iron ($N = 19$)	Placebo ($N = 19$)
Hemoglobin *g/100mL*	12.2 ± 1.0	12.2 ± 1.0
Leukocytes $10^9/L$	6.8 ± 1.4	6.9 ± 2.2
Platelets $10^9/L$	228 ± 69	230 ± 77
Total iron μg/*100mL*	60 ± 25[a]	79 ± 25
Transferrin *mg/100mL*	197 ± 37[a]	171 ± 20
Transferrin saturation%	22.81 ± 10.82[a]	33.33 ± 11.57
Ferritin μg/*L*	140 ± 103	202 ± 100
Redox-active iron μ*mol/L*	0.22 ± 0.04	0.21 ± 0.03
NTBI μ*mol/L*	28.5 ± 83.7	74.1 ± 131.1
Bicarbonate *mmol/L*	23.9 ± 2.3	23.2 ± 1.8
PTH *pg/mL*	330.8 ± 303.4	367.0 ± 238.8
Ca×P $mmol^2/L^2$	4.68 ± 0.95[a]	4.02 ± 0.63
Homocysteine μ*mol/L*	25.4 ± 5.9	25.0 ± 8.9
C-reactive protein *mg/100mL*	0.85 ± 0.49	0.68 ± 0.25
Albumin *g/L*	35.8 ± 4.3	36.4 ± 3.9
Total cholesterol *mg/100mL*	212 ± 57	183 ± 42
LDL cholesterol *mg/100mL*	136 ± 49[a]	104 ± 40
HDL cholesterol *mg/100mL*	44 ± 14	50 ± 16
Triglycerides *mg/100mL*	189 ± 135	163 ± 110

NTBI, non–transferrin-bound iron; PTH, parathyroid hormone; Ca×P, calcium × phosphorous ion product calculated as serum calcium (mmol/L) × serum phosphate (mmol/L). Mean ± SD.

[a] $P < 0.05$ vs. placebo.

Iron status

At baseline, total iron was lower in the iron group. As expected, total iron increased after infusion of iron sucrose, Δtotal iron was 601 μg/100 mL (95% confidence interval CI 507, 696; $P < 0.01$) compared to 0 μg/100 mL (CI −5, +5) in the placebo group ($P < 0.01$ vs. iron group). Transferrin (TRF) was higher and transferrin saturation (TSAT) lower in the iron group at baseline (Table 2).

In patients with iron infusion, ΔTRF was −16 mg/100 mL (CI −27, −5; P = NS) and in patients with placebo −2 mg/100 mL (CI −11, 7; P = NS) ($P < 0.05$ between groups). Since calculated TSAT after intravenous iron administration reflects both transferrin-bound iron and iron bound to circulating iron agent, we did not include ΔTSAT in our results. Ferritin levels were not different between groups before or after iron infusion. Within the iron group, ferritin levels increased slightly, with Δferritin of 16 μg/L (CI 2, 30; P = NS) versus Δferritin −4 μg/L (CI −17, 8; P = NS) in the placebo group. Non–transferrin-bound iron was not different between groups at baseline, ΔNTBI was 237.2 μmol/L (CI 173.6, 300.8; $P < 0.01$) after administration of iron sucrose, compared to –18.8 μmol/L (CI −74.2, 36.3; P = NS) after placebo ($P < 0.01$ between groups) (Fig. 1). Levels of redox-active iron were not different at baseline, Δredox-active iron was 0.76 μmol/L (CI 0.54, 0.98; $P < 0.01$) in the iron group, compared to −0.005 μmol/L (CI −0.02, 0.01; P = NS) in the placebo group ($P < 0.01$ vs. iron group) (Fig. 2).

Basal forearm blood flow

Mean basal blood flow in both forearms before infusions was 3.81 mL/min/100 mL (CI 2.94, 4.68) in the iron and 3.12 mL/min/100mL (CI 2.64, 3.61) in the placebo group (P = NS between groups) (Fig. 3). After infusion of 300 mg iron sucrose basal FBF was 3.73 mL/min/100 mL (CI 3.04, 4.42), which was 59% higher than the 2.34 mL/min/100 mL (CI 1.94, 2.75; $P < 0.05$ vs. baseline, $P < 0.01$ vs. iron) after placebo infusion. ΔFBF

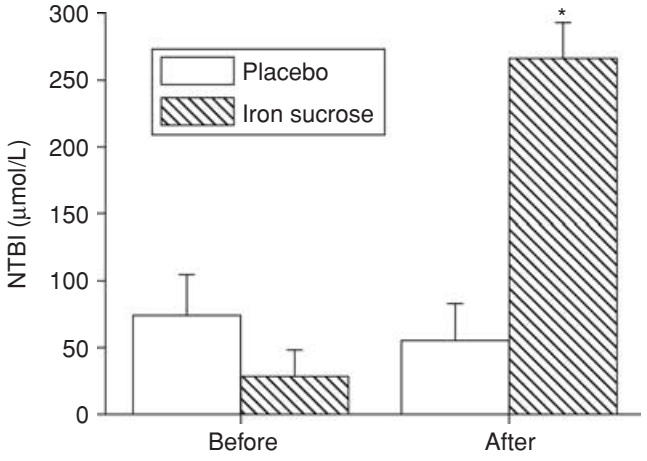

Fig. 1. Non-transferrin bound iron (NTBI, µmol/L) before and after infusion of 300 mg of iron sucrose over two hours (hatched columns, $N = 19$) or placebo (open columns, $N = 19$). Mean ± SEM. $^*P < 0.01$ vs. baseline and between groups.

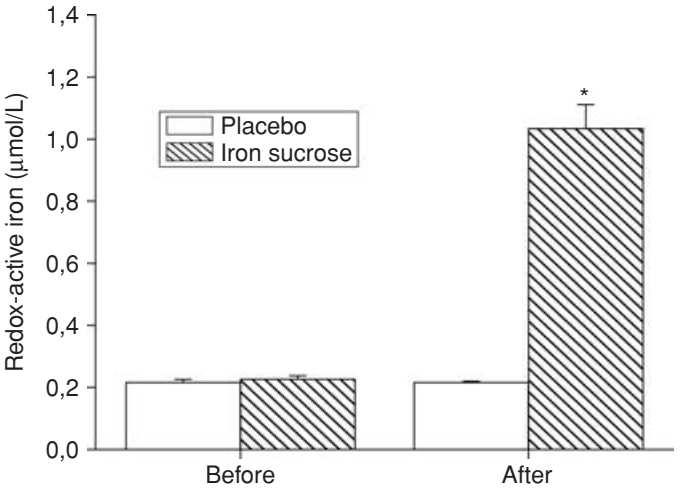

Fig. 2. Redox-active iron (µmol/L) before and after infusion of 300 mg of iron sucrose over two hours (hatched columns, $N = 19$) or placebo (open columns, $N = 19$). Mean ± SEM. $^*P < 0.01$ vs. baseline and placebo.

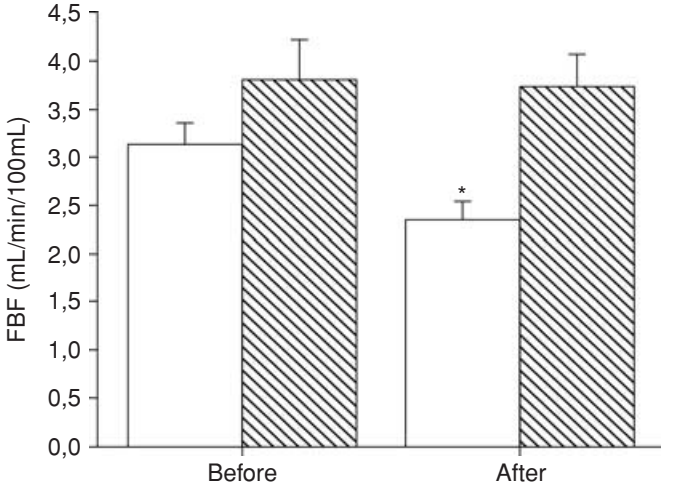

Fig. 3. Basal forearm blood flow (FBF) before and after infusion of 300 mg of iron sucrose (hatched columns) or placebo (open columns). Mean ± SEM. $^*P < 0.05$ vs. baseline, $P < 0.01$ vs. iron.

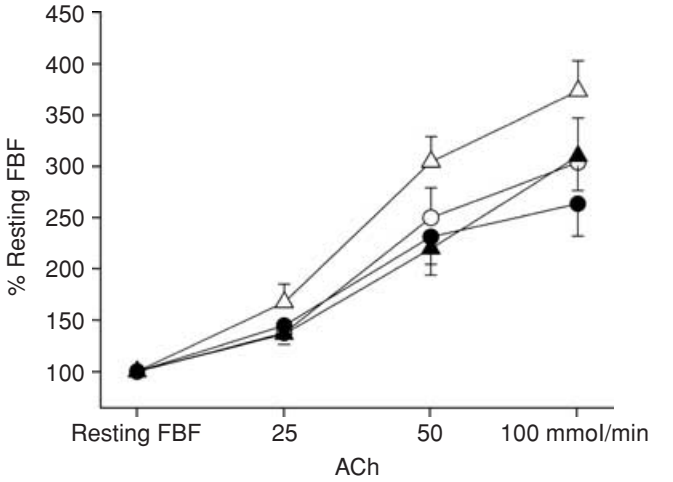

Fig. 4. Forearm blood flow (FBF) response (% of resting FBF) to incremental doses of intra-arterial acetylcholine (ACh) before infusion of iron sucrose (300 mg, filled circles) or placebo (open circles), and after infusion of iron sucrose (filled triangle) and placebo (open triangle). Mean ± SEM.

was −0.11 mL/min/100 mL (CI −0.70, 0.49) in the iron and −0.78 mL/min/100 mL (CI −1.29, −0.26) in the placebo group ($P = 0.08$ between groups, t test; P = NS, Bonferroni corrected post-hoc test).

Forearm blood flow reactivity

Acetylcholine caused a dose-dependent increase in FBF to a maximum of 263 ± 32% and 304 ± 43% before iron or placebo infusion, respectively (P = NS between groups). Responses were comparable after drug infusions, with 310 ± 33% after iron sucrose, and 373 ± 29% after placebo (P = NS vs. baseline and between groups) (Fig. 4).

The endothelium-independent vasodilator GTN increased resting FBF to 232 ± 22% and 234 ± 18% in the iron or placebo groups, respectively (P = NS between groups). Iron or placebo had no effect on GTN-induced vasodilation, FBF increased to 258 ± 21% and 270 ± 30%, respectively (P = NS vs. baseline and between groups) (Fig. 5).

The NO-synthase inhibitor L-NMMA decreased resting FBF to 70 ± 4% and 74 ± 4% before iron or placebo infusions, respectively (P = NS between groups). Again, iron or placebo infusion did not influence vasoconstriction to L-NMMA, which decreased FBF to 72 ± 3% and 73 ± 4%, respectively (P = NS vs. baseline and between groups) (Fig. 6). Absolute changes in FBF in response to

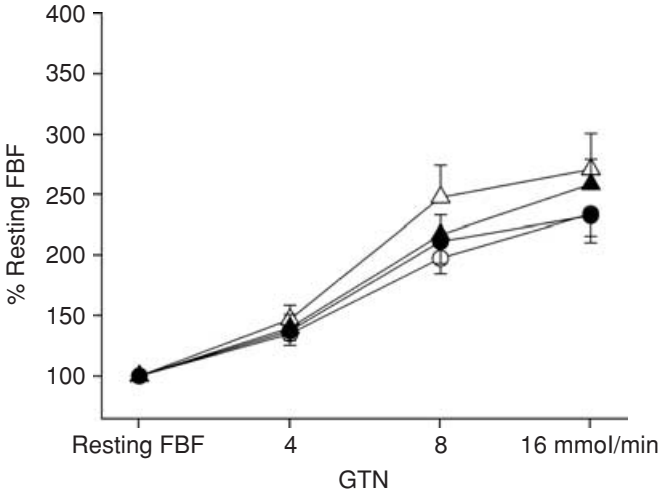

Fig. 5. Forearm blood flow (FBF) response (% of resting FBF) to incremental doses of intra-arterial glycerol-trinitrate (GTN) before infusion of iron sucrose (300 mg, filled circles) or placebo (open circles), and after infusion of iron sucrose (filled triangle) and placebo (open triangle). Mean ± SEM.

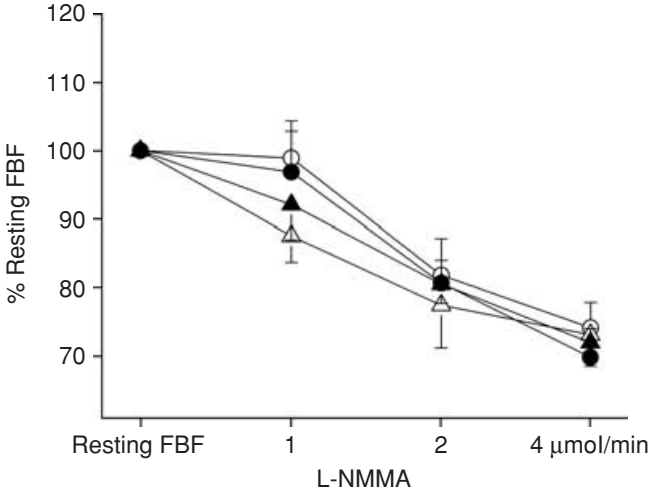

Fig. 6. Forearm blood flow (FBF) response (% of resting FBF) to incremental doses of intra-arterial L-N-mono-methyl-arginine (L-NMMA) before infusion of iron sucrose (300 mg, filled circles) or placebo (open circles), and after infusion of iron sucrose (filled triangle) and placebo (open triangle). Mean ± SEM.

acetylcholine, GTN, and L-NMMA in the intervention arm were also not different between groups (data not shown).

Adverse events

Study-related adverse events included paresthesia in the extremities during FBF measurements ($N = 4$ in the iron, $N = 3$ in the placebo group). Two patients per group reported pain at the puncture site; one patient in the iron group developed thrombophlebitis. One patient in each group reported cephalea. There was no difference in local or systemic adverse events between the two groups.

DISCUSSION

In this prospective, randomized, placebo-controlled, double-blind trial we found a significant increase of total iron, NTBI, and redox-active iron in renal failure patients after intravenous administration of 300 mg iron sucrose over two hours. After iron infusions basal FBF was 59% higher than after placebo administration. In contrast, no effect of iron sucrose on vascular reactivity, an early indicator of vascular disease, was detectable.

High-dose intravenous iron supplementation is an established adjuvant therapy for renal anemia and results in a decrease of required erythropoietin doses [2, 3, 6, 19]. However, concerns have been raised about a possible increase in cardiovascular disease risk associated with intravenous iron therapy. Data from some cohort studies show a correlation between increased body iron stores and progression of atherosclerotic lesions, risk of acute myocardial infarction, and cardiac death [20, 21]. Evidence points to an increased cardiovascular disease risk in carriers of a hemochromatosis gene mutation (*HFE* C282Y) [22], while blood donation has been associated with reduced risk [23]. Endothelial function as an early indicator of vascular disease is impaired in conditions with iron overload, such as thalassemia and hemochromatosis [24, 25]. Conversely, improvement of endothelial function has been described after application of desferrioxamine in coronary heart disease [26], as well as after phlebotomy in hemochromatosis patients [25].

Several mechanisms of the detrimental effect of iron on the vasculature have been suggested. Labile iron leads to increased generation of reactive oxygen species and results in reduced NO availability and endothelial dysfunction. Impaired NO availability might be caused by accelerated lipid peroxidation [10] or direct inactivation by NTBI [11, 27]. Furthermore, iron uptake into endothelial cells via transferrin receptors plays an important role in hydroperoxide-induced intracellular oxidative stress. Conversely, intracellular oxidative stress, caused by glutathione depletion, triggers transferrin receptor overexpression, increases the intracellular labile iron pool, and results in endothelial cell apoptosis [28].

However, only few in vivo data have been published about the relationship between intravenous iron therapy, oxidative stress, and endothelial function. Rooyakkers et al [29] reported a significant decrease in forearm reactive hyperemia in healthy volunteers after infusion of 100 mg of iron sucrose, accompanied by an increased generation of superoxide.

In renal failure patients the relationship between body iron status and vascular disease is even less well defined [30]. In these patients, oxidative stress is increased due

to both the uremic condition and a lower concentration of antioxidants [31–33]. After intravenous infusion of 100 mg of iron sucrose, a significant increase of transferrin saturation, NTBI, and malondialdehyde, as well as a decrease of plasma superoxide dismutase activity, has been reported in chronic kidney disease and HD patients, suggesting exacerbation of oxidative stress by intravenous iron [12, 34–37]. The infusion of antioxidants, such as vitamin E or melatonin, reduces oxidative stress after intravenous iron therapy in dialysis patients [12, 38]. An observational study in HD patients has shown an association of carotid artery intima-media thickness with cumulative intravenous iron dose, serum ferritin, as well as concentration of advanced oxidation protein products [39], indicating a potential role of iron-induced oxidative stress in early stages of atherosclerosis. However, because of the cross-sectional design, this study does not prove a causal relationship between long-term intravenous iron therapy and vascular disease. The other above-mentioned studies only examined serum markers of iron-induced oxidative stress, and there are no such data available for PD patients.

In the present study, basal FBF after infusion of iron sucrose was higher than in the placebo group. Thus, it has to be considered that iron substitution exerted a vasodilatory response in the forearms. In animal studies, release of free iron from intravenously administered iron preparations has been found to induce hypotension, which has been attributed to a temporary suppression of adrenergic vasomotor tone [40]. However, it is difficult to extrapolate animal data to humans. No systemic vasodilation was detectable in our cohort, as evidenced by a lack of changes in systemic blood pressure or pulse rate (data not presented). Furthermore, the change in basal FBF (ΔFBF) after iron or placebo infusion did not reach level of significance between groups. Nevertheless, a vasodilatory effect of iron might have been counteracted by systemic homeostatic mechanisms. This is also in agreement with a previous study, where systemic hypotensive reactions to intravenous iron sucrose infusion were dose-dependent and mainly observed after doses higher than 300 mg [6].

In contrast to basal FBF, vascular reactivity is considered an indicator of early vascular disease. The lack of association between iron infusion and blood flow reactivity in our trial compared to previous studies is an interesting finding. We used a prospective, randomized, placebo-controlled, double-blind study design to examine the association between iron therapy and resistance artery vascular function in dialysis patients, whereas in the previously mentioned study by Rooyakkers et al [29], conduit vessels in healthy subjects were assessed under unmasked time-control conditions. In dialysis patients, endothelial dysfunction has been described [41, 42]. However, in our cohort of unselected PD patients FBF was similar to that seen in healthy subjects [43]. This is in line with other findings, where arterial wall distensibility was impaired in HD, but not in PD patients [44]. These differences might be attributed to lower comorbidity in PD patients [45], different medication, or iron status between dialysis patient populations. In contrast to the study by Rooyakkers et al [29], we have used a higher dose of iron sucrose (300 vs. 100 mg), but it was administered with a slower infusion rate (2.5 mg/min vs. 5 mg/min). Despite the lack of a detrimental influence of 300 mg of iron sucrose on vascular reactivity in our study, we found a marked increase of total iron and NTBI compared to the placebo group. NTBI is a heterogenous mixture of complexes, and it is likely that its composition varies with both extent and type of iron overload [46]. For deleterious effects on endothelial cells, the availability of redox-active iron, which is only a part of NTBI, probably is more important than total body-iron stores or dose of iron. In the present study, we also found a significant increase of redox-active iron after administration of iron sucrose. However, concentrations were smaller than those reported in thalassemia patients [18]. Therefore, our data strongly suggest that after infusion most iron which can be detected by the mobilizer-dependent NTBI-assay is bound to serum components and is redox-inactive, thus not affecting vascular reactivity. In this context, citrate and albumin are discussed as possible ligands of NTBI [46].

There are some limitations to this study. We did not find an acute effect of iron sucrose on forearm blood flow reactivity. However, a possible long-term effect of larger intravenous iron doses on vascular function and cardiovascular morbidity of dialysis patients cannot be excluded. Furthermore, since measurements of FBF reactivity were conducted immediately after infusions, delayed effects of intravenous iron cannot be ruled out. The power of this study was calculated based on an expected difference in vascular function between iron and placebo infusion of at least 75%. The patient number may have been too low to detect smaller differences between the two groups. However, the almost identical forearm blood flow dose-response curves (see Figs. 4–6) make even such smaller differences between iron and control group patients unlikely. The gold standard strain-gauge plethysmography was used to measure vascular function in this study. We have previously demonstrated that drug reactivity to ACh and GTN does not depend on basal FBF in this model [15]. Therefore, a minor change in forearm basal vascular tone does not influence our finding on unaltered vascular reactivity in subjects with PD. Of note, absolute changes in FBF in response to the vasoactive substances were also not different between groups.

Despite careful randomization, serum-iron and transferrin saturation at baseline were slightly lower in the iron group compared to the control group. However, the marked increase of iron after infusion of 300 mg of iron

sucrose should have been appropriate to show effects on vascular function if present, even when supposing a higher buffering capacity for iron by transferrin in this group. Finally, vascular reactivity was subject to diurnal variability, with higher vasodilatory responses to Ach and GTN after infusion of iron or placebo, respectively. However, there was no difference detectable between randomly allocated patient groups.

CONCLUSION

A single intravenous infusion of 300 mg of iron sucrose did not impair FBF reactivity as measured by plethysmography in dialysis patients, despite a marked elevation of total iron and NTBI, as well as a significant increase of redox-active iron. However, basal FBF was increased by iron sucrose and the possible impact of iron-induced vasodilation has to be considered, especially when higher doses of iron sucrose are infused. Prospective trials are needed to study long-term influences on cardiovascular mortality in patients requiring high-dose intravenous iron therapy.

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